

The University of Chicago

CHANGES IN THE TEETH FOLLOWING
HYPOPHYSECTOMY

I. CHANGES IN THE INCISOR OF THE
WHITE RAT

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

By
ISAAC SCHOUR

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE AMERICAN JOURNAL OF ANATOMY
Vol. 50, No. 3, 1932

The University of Chicago

CHANGES IN THE TEETH FOLLOWING
HYPOPHYSECTOMY

I. CHANGES IN THE INCISOR OF THE
WHITE RAT

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

By
ISAAC SCHOUR

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE AMERICAN JOURNAL OF ANATOMY
Vol. 50, No. 3, 1932



CHANGES IN THE TEETH FOLLOWING HYPOPHYSECTOMY

I. CHANGES IN THE INCISOR OF THE WHITE RAT¹

I. SCHOUR

SIX PLATES (TWENTY-SEVEN FIGURES)

INTRODUCTION

The purpose of this study was to investigate the effects of hypophysectomy on the rat incisor. This tooth is advantageous for experimental studies because of its relatively rapid eruption and its persistent growth. It permits a study in a single adult of the structural changes which some of the dental cells and tissues undergo from early development to maturity.

The animals were hypophysectomized by and cared for in the laboratory of one of us (H.B.v.D.); the rest of the work and the writing of the paper were done by the other author (I.S.). We are deeply indebted to Dr. G. W. Bartelmez, who directed this investigation and who helped very generously in the carrying out of the various phases of the work. We are thankful to Dr. R. R. Bensley and Dr. F. B. Noyes for their encouragement and suggestions.

REVIEW OF LITERATURE

A careful survey of the literature indicates that no previous gross and histologic study of incisors of hypophysectomized rats has been made. The teeth of hypophysectomized dogs, however, have been studied. Ascoli and Legnani ('12)

¹ This work was in part supported by the John D. Hertz Fund of The University of Chicago. Published under the co-authorship of I. Schour and H. B. van Dyke in The American Journal of Anatomy.

reported that hypophysectomy in a dog resulted in an abnormal persistence of the deciduous teeth. Kranz ('14) studied the teeth of a hypophysectomized dog and its control which he obtained from Ascoli and found a few small differences in chemical composition and a considerable difference in size. Microscopic studies revealed no histologic differences. Aschner ('12) observed in a hypophysectomized dog the eruption of the permanent incisors and cuspids in spite of the persistence of the deciduous teeth so that there arose a double row of teeth. Downs ('30) reported a delayed eruption and a crowding and 'flaring' of the teeth in hypophysectomized dogs. His histologic findings, like those of Kranz, were negative.

Clinical literature contains many references to variations in dentition and dental anomalies as the result of pituitary changes. Recently considerable literature has developed attempting to associate so-called pyorrhea with disturbances of the anterior lobe of the hypophysis. However, because of the difficulties involved in finding the exact nature of the pituitary disturbances and in eliminating the possible effects of other concomitant disturbances and because of the lack of a detailed study of teeth in many of these cases, these references are of less positive value than one would desire.

MATERIAL AND METHODS

This study is based on twenty-three successfully hypophysectomized rats. Their age when operated upon ranged from thirty-six to sixty-four days and the interval between the operation and the death of the animal ranged from 63 to 459 days (table 1). These animals were part of the series used by one of us in his investigations on the function of the hypophysis (van Dyke and Lawrence, '30; Wallen-Lawrence and van Dyke, '31). An operation was considered successful when there was no spontaneous growth and no spontaneous oestrous cycle for weeks or months. In all cases in which detailed necropsies were made, there were found marked atrophy of the gonads and the external genitalia and no

remains of the hypophysis. Smith ('30), who in an extensive study made serial sections of the entire pituitary region of hypophysectomized rats, could correlate complete removal of the gland with functional impairment in respect to cessation of growth and disappearance of oestrous cycles. We, therefore, felt that we could accept the functional evidence

TABLE 1

Table of twenty-three successfully hypophysectomized rats arranged in order of postoperative life¹

NO. OF ANIMAL	AGE IN DAYS AT OPERATION	KILLED OR DIED	AGE IN DAYS AT DEATH	INTERVAL IN DAYS BETWEEN OPERATION AND DEATH	GROUPING ACCORDING TO HISTOLOGIC FINDINGS IN INCISORS ²
289	55	Died	118	63	I
324	45	Killed	141	96	I
319	47	Killed	155	108	3
314 ⁴	46	Died	156	110	I
265	50	Died	195	145	I
264	49	Died	216	167	I
317	46	Killed	213	167	I
257	64	Died	242	178	II
199	49	Died	243	194	II
240	47	Died	254	207	I
221	44	Died	263	219	I
268	61	Killed	304	243	I-II ⁵
228	45	Died	296	251	III
144 ⁴	41	Died	298	257	II
108	36	Died	309	273	III
154	37	Died	329	292	III
216	39	Died	411	372	III
151	40	Died	420	380	III
258	64	Killed	476	412	II-III ⁵
186	50	Killed	478	428	III
156	41	Killed	473	432	III
111	40	Died	483	443	III
113 ⁴	36	Killed	495	459	III

¹ A hypophysectomy was considered successful when there was no spontaneous growth and no spontaneous oestrous cycle for weeks or months.

² The x-ray findings were positive in each case.

³ Rat 319 was not studied histologically, because it was reserved for skeleton preparation.

⁴ Selected for detailed histologic description as representative of a respective group.

⁵ Belong to border line of respective grouping, possibly because these animals received sixty-one injections of growth hormone which may have retarded the development of histopathologic changes.

as a reliable criterion of the success of the operation without the preparation of serial sections of the hypophyseal region.

A study was also made of sixteen litter-mate controls, most of which were unsuccessfully hypophysectomized animals (table 2). The latter as well as the unoperated litter-mate controls showed no indication of hypophyseal deficiency. We accept this fact as evidence that the changes noted in the

TABLE 2

Table of sixteen litter-mate controls, most of which were unsuccessfully hypophysectomized¹

NO. OF RAT	LITTER-MATE CONTROL TO	KILLED OR DIED	AGE IN DAYS AT DEATH
323	324	Killed	141
320 ²	319	Killed	155
312	314	Killed	176
318	317	Killed	213
202	199	Killed	253
238	240	Killed	257
148	144	Died	279
270	268	Killed	304
219	221	Killed	305
168	166	Killed	364
155	151, 152, 154	Killed	370
146	144	Died	377
112	108, 111, 113	Killed	394
157	156	Killed	473
255	257, 258	Killed	476
214	210, 216	Killed	561

¹ The histologic and radiographic findings were normal in each case.

² Rat 320 was not studied histologically because it was reserved for skeleton preparation.

teeth of the successfully operated group were not due to operative injuries.

A study was also made of six rats which had been operated upon and in which the weight curves and the oestrous cycles possibly indicated only a partial hypophysectomy (table 3).

The operations were performed by the parapharyngeal approach following the technique of Prof. Philip E. Smith.

In order to measure the rate of eruption, a horizontal marking with a fine file (Swiss jewelers' screw-head file) was

made near the gingival line along the distolabial margin of the upper incisor. With an adjustable caliper provided with fine points the distance was measured between the point where the marking crossed the distolabial margin and the point where the surface of the gingivae crossed the same margin. This distance was read in twentieths of a millimeter with the aid of a stage micrometer and a dissecting microscope. The distance between the same points mentioned above was read seven days later. The difference between the two readings represented the rate of eruption for that period.

TABLE 3

Table of six rats that were hypophysectomized with only partial success¹

NO. OF ANIMAL	AGE IN DAYS AT OPERATION	KILLED OR DIED	AGE IN DAYS AT DEATH	INTERVAL IN DAYS BETWEEN OPERATION AND DEATH
230	47	Killed	301	254
175	47	Died	348	301
166	34	Died	364	330
184	49	Died	459	410
152	40	Killed	471	431
210	42	Killed	561	519

¹ The partial success of the hypophysectomy was evidenced by an intermediate disturbance in oestrous cycles and an irregular weight curve. The histologic picture in this series was as a whole normal except for some minor disturbances, observed in three animals, which were connected with the epithelial sheath. Apparently most if not all of the changes that may have been produced originally following the operation were no longer recorded in the histologic sections because of the length of the postoperative life.

New marks had to be made each week in the normally erupting teeth.

The upper incisors of six sets of hypophysectomized animals and their litter-mate controls were x-rayed weekly while under observation. Dental x-ray films were used that required an exposure of only three-fourths of one second, so that the animals did not need to be anesthetized. An effort was made to use a constant angle of exposure.

The animals were fixed with 5 per cent neutral formalin or formol-bichromate immediately after being killed or as soon as they were found dead. The head was severed and a

midsagittal section was made to facilitate the x-raying of the teeth. All of the factors of x-raying were kept as constant as possible. The further dissection consisted of separating the mandibles and separating the upper incisor from the upper molar group. In most instances the upper right incisor, the upper right molars, and lower right mandible were prepared for histologic study, while the remainder of the jaws and teeth were kept in the fixative for further gross study and ground-section preparation, or dried for chemical analysis.

The fixed teeth with their investing tissues were washed, decalcified in 5 per cent nitric acid followed by 5 per cent sodium sulphate, embedded in celloidin, and stained with hematoxylin and eosin or with iron-hematoxylin. The sections were mounted in serial order employing Maximow's modification of the Russian method (Romeis, '28).

Ground sections of only the hard tissues of the teeth were prepared by grinding on a carborundum wheel driven by a motor. Ground sections of both the hard and soft tissues of the teeth were prepared by first embedding in balsam (Marshall, '27) or in celloidin and then grinding with the grinding machine devised by G. V. Black ('24). A simpler method of preparing ground sections of both hard and soft tissues consisted of first embedding the tooth with its investing tissues in balsam or celloidin and then grinding it by hand only in the central position. The ground surface was then polished and studied under fluid by reflected light.

The radiographs of the dissected halves of the heads of a number of the animals were magnified three times and used for planimeter readings of the total sagittal surface area of the teeth and the sagittal surface area of the calcified portion. The difference between the two readings gave the approximate sagittal surface of the pulpal portion.

GROSS FINDINGS

Findings in the living animals

In the living hypophysectomized animal the exposed portions of the incisors are seen to be considerably smaller than those of the litter-mate controls. The contour of the exposed portion is normal except in some of the oldest animals where waves of enamel are readily seen with the naked eye (fig. 1).

The rate of eruption is retarded within a week following the operation. This retardation is progressive. In the oldest animals the eruption has ceased entirely (fig. 1). That this disturbance in eruption is a result of the hypophysectomy can be readily proved by replacement therapy. Upon the injection of an extract of the anterior lobe of the hypophysis the eruption is accelerated if treatment is begun early enough (Schour and van Dyke, '32).

The radiographs of the living as well as the dissected animals show striking changes in form and contour in all cases except in the early stages.

Radiographic findings

We shall first describe some of the salient points regarding the radiograph of the normal mature rat incisor (figs. 4, 7, 12). The x-ray of the normal rat incisor presents a longitudinal section of a curved and partially hollow cylinder with an incisal edge that has a long and slightly concave bevel on the lingual surface. The shadow of the tooth is intense and more or less uniform in approximately its anterior third or half and has a sharply delineated external border. Beginning with the middle third, because of the increasingly large size of the pulp, this shadow splits into a convex and a concave border which taper as they proceed posteriorly and which finally end hair-like at the most basal end. The convex labial border of the shadow represents enamel and dentin; the concave lingual border represents dentin and a comparatively slight amount of cementum (Toyofuku, '11).

The curve of the tooth is perfectly smooth and represents in the upper incisor a greater segment (210°) of a smaller

circle and in the lower a smaller segment (140° to 145°) of a larger circle (Owen, '45; Addison and Appleton, '15). The radiographs of the right and left incisors of an animal are symmetrical.

The x-ray of the incisor of the hypophysectomized rat that has lived six months or more after hypophysectomy (figs. 3, 6, 13) presents a longitudinal section of an irregularly curved and for the most part solid cylinder with an incisal edge that has a short and considerably concave bevel on the lingual surface. The shadow of the tooth is intense and uniform within approximately its anterior eight- or nine-tenths where there is relatively little pulp present. This shadow begins to split at a much more posteriorly situated level than in the normal. The concave border does not taper gradually, but rather abruptly, while the convex border usually shows a series of irregular foldings. There are thus two areas to be distinguished in the radiographs and, as we shall see later also in the histologic sections, an anterior zone which comprises about eight- or nine-tenths of the tooth and a basal zone which contains the base of the pulp and the seat of activity of Hertwig's sheath. The extent of the shadow shows that the tooth substance is practically solid in the anterior zone. The labial contour in the anterior zone is often interrupted by a varying number of waves or indentations and does not represent a smooth arc of one circle, but is often composed of two arcs of two different circles. The anterior arc usually belongs to a smaller circle and represents the older portion of the tooth, while the posterior arc is flatter and represents the younger portion of the tooth (fig. 9). In rat 151 the curvature is so distorted that the anterior and posterior fourths are almost parallel (fig. 8). In the basal zone the labial contour is distorted and presents multiple foldings. The radiograph of the incisor of the hypophysectomized rat that lived a given period after the operation gives a constant pathognomonic picture.

The radiographs of the right and left incisors of the same hypophysectomized animal are usually not symmetrical. The

x-ray of the incisor of the experimental animal differs according to the length of the postoperative life. The differences, however, are only in the degree of the intensity of the changes, except in the earlier stages. The shortest interval which we have so far found to register radiographic changes is sixty-three days postoperatively in rat 289. In some cases the multiple folding is not merely concentrated in the basal zone, but is found to be distributed along the entire labial surface (fig. 5).

The planimeter readings of the radiographs show that the incisors of the hypophysectomized animals of long standing are about two-thirds the area of their litter-mate controls, and yet the amount of hard dental tissues is about the same in the experimental and control animals. There is no discrepancy between these facts because the hard tissues, at least in parts, persist at the expense of the pulp which is being gradually obliterated.

Photographs of dissected portions of the jaws corroborate the planimeter findings in regard to comparisons in size (fig. 2).

HISTOLOGIC FINDINGS

Histology of incisor of normal rat

Before presenting our histologic findings in the experimental animals, we shall consider a few of the outstanding points regarding the histology of the normal rat incisor. The histology of the normal rat incisor is a problem in itself and a detailed discussion of it will not be undertaken here.

The normal rat incisor has no root and consists of a powerful crown of dentin that is covered with enamel on its convex surface and with cementum on its concave surface (fig. 14). Its enamel organ forms at the basal end of the tooth the epithelial sheath of Hertwig which persists throughout life and which contributes to, guides, and makes possible the continuous growth of the incisor. The enamel organ proper persists structurally and functionally only over the convex surface. At the basal end it has fundamentally the same four layers as in man. However, over the greatest extent of the

convex surface it consists chiefly of the inner layer of high columnar ganoblasts and of the outer layer of cuboidal and polyhedral cells that are arranged in papillae and are surrounded by a very abundant capillary blood supply. This layer has been called the papillary layer (fig. 18).

Toward the more anterior portion of the tooth, the ganoblasts become shorter and the papillae become lower. The formerly highly specialized enamel organ becomes reduced and shows a gradual transition to the stratified squamous epithelium of the gingivae.

The enamel starts at the base and increases in thickness and becomes fully calcified distally so that here it can be preserved only in ground sections (fig. 21), since no traces of the tissue are left in decalcified preparations. Normally, it follows the convex curvature of the tooth and slightly overlaps the lateral surfaces (Schour, '32).

The dentin increases anteriorly in thickness with a corresponding reduction in size of the inclosed richly vascular pulp. At the anterior end the pulp is normally necrotic and becomes exposed as it is worn down. The cementum is very thin (about 3μ). The periodontal membrane and the labial alveolar periosteum have an exceedingly rich blood supply.

In interpreting histologically the teeth of rats used for experimental purposes one must keep in mind the normal range of variability which may be surprisingly wide. Even with the so-called adequate diet there is a certain range of imperfect calcification and there may be an incidence of caries which may be seriously misleading if considerable control tissue is not studied with the same diligence as the experimental material.

Histopathology of the incisors of the hypophysectomized rat

From the histologic point of view, our material may at present be divided into three groups:

Group I presents the changes found in animals which lived from two to six months after the operation. These changes will be referred to as early changes.

Group II presents the changes found in animals which lived from six to ten months after the operation. These changes will be referred to as intermediate changes.

Group III presents the changes found in animals which lived from ten to fifteen months after the operation. These changes will be referred to as the advanced changes.

There is of course some overlapping in these groups. The histologic studies were made without any reference to the length of time that elapsed between the operation and the death of the animals. When the grouping based on the histologic findings was compared with the postoperative time a definite correlation was readily recognized (table 1).

We have, in addition, four animals which died within two weeks following hypophysectomy and the report on which cannot yet be made.

Group I. We shall present the findings in rat 314 as illustrative of group I. This animal was operated upon when forty-six days old and lived 110 days after the operation. The x-ray of the upper incisors (fig. 9) shows that the contour of the labial surface is composed of two segments, an anterior larger segment of a smaller circle and a posterior smaller and flatter segment of a larger circle. There is a sharp indentation of enamel at the anterior limit of the posterior third of the labial surface of the right upper incisor. In the basal zone there is a band of young enamel which is characterized by its deep staining quality and its persistence in the histologic section in spite of decalcification. The extent of this enamel is shorter and narrower than normal. Its surface is generally even, but occasional enamel globules are found projecting on the surface. Next to the distal end of the ganoblasts are collections of cellular débris with cells of inflammation. The enamel space is clear and suggests the presence of normal enamel except where it is interrupted by a folding of enamel epithelium which almost meets the dentin (fig. 11). This is the same folding which we have seen very distinctly in the x-ray (fig. 9).

The dento-enamel junction undergoes in this region a corresponding but less abrupt folding. On either side of this epithelial invagination the enamel space is filled with organic remains showing poor calcification.

The enamel epithelium shows striking disturbances. At the basal end next to Hertwig's sheath, the epithelium shows a double folding with vicarious enamel formation in form of isolated amorphous enamel drops. Where the epithelium is not found folded, it is disrupted.

About 1 mm. from the most posterior end, the enamel epithelium approaches a normal appearance in some respects. But the arrangement of the papillae and ganoblasts is not always parallel (fig. 19). The cellular débris which is found next to the distal end of the ganoblasts occasionally contains globules of various sizes of eosin-staining material and also some large enamel globules. Some of the cells of the papillae show karyorrhexis. More anteriorly, the papillae become longer and thinner, uneven in width, and end in knob-like enlargements which occasionally bud fork-like (fig. 16). The ganoblasts are still columnar.

Following the epithelial infolding, the papillae become irregular in length and farther apart (fig. 17). They lose their parallel arrangement. Some of their cells show autolysis and karyolysis. The ganoblasts become low cuboidal and squamous and are in some places bordered by a streak of organic remnants of enamel.

Finally, the papillae disappear and a band of two or three layers of irregularly arranged epithelial cells is all that remains of the highly organized enamel organ. At the level of the alveolar crest even this epithelial band is gone and only a few flattened connective-tissue cells border the enamel surface.

The dentin contains many interglobular spaces, but as a whole its structure and calcification are within the normal range. The odontoid layer is prominent at the basal end of the tooth where the new dentin formation proceeds. The odontoblasts appear for the most part normal in shape and

structure. The pulp at the basal end is richly cellular. The protoplasmic processes are normal, but the nuclei appear pyknotic. The intercellular substance looks fibrinous. Toward the incisal edge the pulp assumes the form of a slit and consists of pulpal remains which contain spherical calcified bodies that are characterized by a more intensely calcified periphery. On the lingual side there is an accumulation of dark eosin staining fluid next to Hertwig's sheath.

The labial alveolar periosteum is more or less infiltrated with cells of inflammation. It is widest at the region of the enamel indentation. Toward the anterior third of the tooth, there is an edematous increase in intercellular substance. At the level of the alveolar crest cells are scarce. The blood supply is limited.

The cementum is thicker than normal ($5\ \mu$).

The lower incisor shows a picture similar to that of the upper one, but to a somewhat less marked degree in respect to the enamel epithelium, dentin, and pulp. There is, however, no indentation of enamel. There is a cyst-like area of eosin staining fluid on the lingual side between the Hertwig sheath and the pulp.

Group II. The findings of rat 144 are illustrative of group II. This animal was operated upon when forty-one days old and lived 257 days after the operation. The x-ray (fig. 13) shows an irregular contour of the labial surface. The histologic picture corroborates this fully (fig. 15).

In the basal zone the enamel shows many variations from normal structure to complete absence. The staining reaction of the enamel matrix is highly variable in different locations. At the basal extremity the surface portion of the enamel matrix shows deep slits. In the posterior third of the labial contour the enamel space is very irregular, as already seen in the x-ray. A number of epithelial and connective-tissue ingrowths reduce the enamel in some places to a very narrow border or allow no enamel to be formed at all. On either side of these epithelial ingrowths the enamel space is filled with organic matrix material of irregular texture and irregular enamel rod direction.

In the anterior two-thirds of the labial contour the enamel space is as a whole free from organic enamel matrix. Toward the basal extremity, the enamel epithelium is folded. The ganoblasts are low columnar, but soon become cuboidal (more anteriorly). The papillae are low and irregularly curved in either the anterior or posterior direction (figs. 22, 23).

The enamel epithelium shows the greatest irregularity near and at the epithelial ingrowths. The subjacent connective tissue of the latter contains enamel globules of various sizes and staining reactions and also peculiar collections of eosin-staining globules and masses. These are irregular in outline and surrounded by streaks that stain like organic enamel matrix and are lined by flat and degenerated epithelial cells. These contents of the connective tissue are either the products of aborted enamel formation or part of the disturbed enamel epithelium. Some the papillae proliferate into the labial alveolar periosteum in form of irregular cords of various widths. The outer portions of some of these become separated from the epithelium which lines the enamel space and are then found in the form of epithelial islands and clusters in the connective tissue of the labial alveolar periosteum (fig. 20). Some of the cells of the papillae and most of the cells of the islands show severe necrobiotic changes in form of autolysis, pyknosis, karyorrhexis, and karyolysis.

As a whole there is little or no enamel epithelium lining the enamel space. In most of the anterior portion of the tooth, the enamel space is, because of the complete absence of enamel epithelium, lined directly by connective tissue or a strand of enamel matrix. Here and there epithelial cells undergoing degeneration are also seen lining the enamel space.

The dentin is for the most part normal in structure. It contains many interglobular spaces, but these may be regarded as being still within the normal range. Many tubular spaces are found which are probably inclusions of pulpal remains. In the basal zone, the dentin on the labial side is completely perforated in some places so that the pulp and the labial alveolar periosteum communicate.

The pulp is small in extent. Its blood supply is relatively scant. The pulp tissue becomes somewhat fibrous in the anterior direction. Its surface is folded. Toward the incisal edge the remains of the pulp tissue shows calcified globules at the periphery.

The blood supply of the periodontal membrane is relatively scant. Toward the base its fibrous character is somewhat diminished. The gingivae at the labial surface are highly inflamed.

The cementum is $14\ \mu$ thick in many areas. This compares with $3\ \mu$ thickness in rat 146 which is the litter-mate control.

The labial alveolar periosteum is everywhere sprinkled with degenerated epithelial cells. Especially along the enamel space in the posterior half a number of degenerated epithelial cells are sometimes clustered in groups or islands that are situated away from the enamel. There are a few projections of bone into the periosteum. The blood supply is relatively scant. In the posterior portion the periosteum loses some of its fibrous character and assumes more of an edematous character. At one place the alveolar bone approaches the enamel space as close as $15\ \mu$.

The most posterior portion of the labial surface of the tooth presents a folding of enamel and dentin which in longitudinal section has roughly the appearance of the crown of a small unerupted tooth (fig. 26). Since this portion is the one that was formed last it gives us a picture of the degree and nature of the functional capacity of this incisor at the time almost immediately preceding the death of the enamel. The dentin is very poorly calcified and the dentinoid layer is wide and irregular. The odontoblasts appear normal. The enamel epithelium is folded and the enamel shows a peripheral portion which takes a deep eosin stain and which contains deep slits, that are also seen in ground sections of corresponding areas.

Group III. The findings of rat 113 are illustrative of group III (figs. 3, 6, 27). This animal was operated upon when thirty-six days old and died 459 days after the operation. Its

findings are so similar to two of its litter mates (108, 111) that were also hypophysectomized that the figures chosen for this group are taken from this litter. Rat 112 is the litter-mate control (figs. 4, 7).

At the basal zone the enamel is very irregular. It is absent in some areas. Its calcification is incomplete and poor. The enamel space is in some places interrupted by connective-tissue projections or ingrowths which approach the dentin surface or are in direct contact with the latter, or even fill in a concave depression of the dentin. These connective-tissue projections are not always lined by enamel epithelium. They often contain enamel globules that are surrounded by crowded epithelial cells. The enamel space on either side of these infoldings contains enamel matrix showing imperfect calcification, just as we have seen in the earlier stages (fig. 11).

The multiple folding at the base consists of about three deep folds which may have minor folds and a final fourth major folding (fig. 27). The latter is formed so that it gives the appearance of a cusp that has recently been formed. In the anterior zone the enamel as a whole is apparently normal in structure except for some areas along its surface where the presence of organic matrix remains in the enamel space suggests imperfect calcification. These remains are sometimes associated with irregularly reduced enamel epithelium or the close approximation of the alveolar bone. In the basal zone the enamel epithelium is found chiefly in reduced form. Low ganoblasts and short papillae are found in the portion of the tooth that was formed last. In the anterior zone the enamel epithelium is entirely absent except for occasional condensations of irregular epithelial cells (fig. 10).

The dentin is for the most part fairly normal in the anterior zone. It contains, however, a number of vascular inclusions. In the basal zone it is very irregular. Here the dentin sometimes encloses cells. The dento-enamel junction is often very irregular. In ground sections, the dentin shows large prominent interglobular spaces in areas where the overlying enamel is particularly disturbed.

In the anterior zone the pulp is obliterated entirely except for a narrow slit which contains hyalinized blood vessels and many calcified globules (fig. 27). At the base the pulp appears almost normal. But its blood supply is diminished. The blood supply of the periodontal membrane is also diminished. The cementum is 11μ thick. The labial alveolar periosteum is narrower than normal and contains projections of bone which come close to the enamel. In one instance the bone is found to be in contact with the enamel (fig. 24).

Hertwig's sheath is displaced on the lingual. Figure 25 shows the course the epithelial sheath must have followed in order to guide the irregular cemental surface of the dentin.

DISCUSSION

Postmortem changes. None of the changes described above can be ascribed to postmortem alterations. Upon comparing the histologic material of the hypophysectomized animals that were killed and immediately fixed (eight in number) with the material from the animals that were fixed after they were dead for a varying number of hours, it was found that the postmortem changes were only slight. A mitotic figure of a fibroblast was observed in the labial alveolar periosteum of rat 314 which died during the night and was fixed next morning.

Rate of eruption. The rate of eruption is retarded within a week following hypophysectomy and may thus be used as an early and reliable indicator of the success or failure of the operation. In the case of those animals for which the rate of eruption of the incisors was measured within a week following hypophysectomy, one of us who made the measurements without knowing the history of the animals was able to determine on the basis of one week's measurement which animals were successfully operated upon. His determination was later corroborated in each case by the other author who had the records of weight and oestrous cycles.

Curvature of the tooth. The disturbed growth of the alveolar bone of the incisor in the hypophysectomized animal

thwarts the tendency of the incisor to flatten its curvature and contributes to the distortion of form. Instead of one curvature like the arc of a single circle, we sometimes find two curvatures: the curvature of the older portion of the tooth which normally would have been worn off entirely and the curvature of the younger portion of the tooth which, if the tooth had erupted at the normal rate, would have been the only curvature present (fig. 9).

Enamel. The enamel at the basal zone shows at first glance some features which indicate hypophysia and some features which suggest an excessive amount of enamel formation (fig. 27). However, we are dealing here with an accumulation of enamel which would have been worn off if eruption had been normal.

Enamel epithelium. The enamel epithelium which is highly specialized in structure and function is disturbed more or less severely depending on the length of the postoperative life. The sequence of changes is fairly regular along the labial surface of a given tooth or from one stage to another. While in the early changes the enamel epithelium is seen to be absent only in the region near the alveolar crest, in the advanced stages it may be absent along the midregion of the tooth or even more posteriorly (fig. 10). As the interval of time following the operation increases, the epithelial disturbances become more severe and more confined to the basal zone. Thus the degree of epithelial disturbance and the location of the latter may be used as a guide to indicate the length of time that elapsed between the operation and the death of the animal.

Dentin. When looking at a histologic section of the incisor of a hypophysectomized rat one might on first glance suppose that one deals with a pathologic increase in dentin formation, since the space normally occupied by the pulp is for the most part dentified and, in addition, dentin is found to be accumulated in irregular folds at the basal end (fig. 27). However, further consideration shows that there is actually no abnormal increase in the amount of dentin formed. In the incisor of the

normal rat dentin grows by apposition at the pulpal surface in form of layers which move anteriorly along the long axis of the tooth and are constantly replaced by similar layers (Erdheim, '11). The balance between the rate of apposition and the rate of the forward movement of these layers is such that in the mature rat the total amount of dentin substance (in a given tooth) remains the same.

In the incisor of the hypophysectomized rat this balance is disturbed because of the more marked retardation of the rate of eruption. The result is that more dentin is being formed than is worn down, so that the dentin substance does not remain constant, but increases in amount. The new dentin layers that are being constantly and successively apposed cannot immediately occupy the position of the already formed and more slowly erupting dentin layers; thus in parts they accumulate at the expense of the pulp. Because of the more limited space at the posterior end of the tooth, the dentin is here folded irregularly.

This disturbance of balance between the rate of apposition and the rate of forward movement of the dentin layers continues throughout the life of the hypophysectomized animal. One might expect that eventually the accumulation of dentin in the face of a progressive retardation and final cessation of eruption would lead to the total obliteration of the pulp and thus to a complete stoppage of dentin formation, since the pulp is the source of dentin formation. But in the case of the animals studied which lived longest after the hypophysectomy, up to 459 days, there is still evidence of activity in dentin formation. There is probably a progressive slowing down in dentin apposition correlated with the progressive reduction in the volume and vascularity of the pulp.

Cementum and periodontal membrane. The histologic picture of a thickened cementum and a narrowed periodontal membrane coincides with that of a non-functioning tooth (Kellner, '31). Thus we have in the hypophysectomized rat fundamentally a tooth in which the hard dental tissues accumulate increasingly while the soft dental tissues undergo a gradual obliteration.

Disturbance of eruption and function. The histologic picture serves to confirm and amplify the gross, x-ray, and eruption findings. In the advanced stages one could deduct from the foldings and bone anchorage seen in the histologic sections that eruption was seriously disturbed even if one knew nothing of the rate of eruption (fig. 24). In the advanced stages we have a tooth which is insignificant in its function or does not function at all. The fact that in those animals where eruption has ceased entirely the exposed portions of the incisors are not shorter than those of other hypophysectomized animals indicates that the opposing incisors did not occlude or wear off. In rat 113, caries was found at the incisal surface of an upper incisor. This could not occur on a surface that was being constantly worn down.

Causal relationship between hypophysectomy and the disturbances in the rat incisor. We feel justified in concluding that the changes in the dental tissues that we have reported result from the hypophysectomy for the following reasons:

1. Animals that were unsuccessfully hypophysectomized and that were therefore exposed to injuries that were quite similar to those incidental to successful hypophysectomy showed a normal dental picture.

2. Replacement therapy accelerated the rate of eruption after it had been retarded following hypophysectomy. The experimental animals that were given a series of injections of the growth-producing hormone at three separated times (rats 258, 268) showed histologic disturbances that were less advanced than those in animals that lived a similar period of time following the hypophysectomy (table 1).

3. The changes differ from those observed in deficient diets (Orban, '27), rickets (Erdheim, '14), parathyroidectomy (Erdheim, '06; Toyofuku, '11; Kranz, '14) and thyroparathyroidectomy (Hammett, '22; Jung and Skillen, '29).

Possible basis for changes following hypophysectomy. Upon studying a series of animals that lived varying intervals following hypophysectomy one is able to trace a sequence of changes that occur more or less regularly and constantly.

It has been a gratifying experience that as more and more material was studied and details were accumulated, they all contributed to a clearer understanding of the process. To follow the various steps in this process has, therefore, not been difficult. On the other hand, the explanation of the process is very difficult and awaits further histologic and experimental investigation. We are dealing, on one hand, with an endocrine organ and, on the other hand, with the tooth, which is the richest calcium containing organ in the body. The fact that all the endocrine organs may be closely interrelated suggests immediately the possibility that in removing the hypophysis we are disturbing the parathyroids which are known to play an important rôle in calcium metabolism and the removal of which is known definitely to disturb tooth development. However, the incisor of the hypophysectomized rat presents as a whole a different picture from that of the parathyroidectomized rat. The incisor of the parathyroidectomized rat shows a pathological opacity. Its rate of eruption is retarded (Gottlieb, '20; Orban, '27), but apparently not as much as in hypophysectomy. The folding of enamel and dentin is comparatively mild. The chief change is in the dentin which shows a wide dentinoid border and which therefore weakens the tooth so that fractures are common (Erdheim, '07; Toyofuku, '11). Following hypophysectomy, the dentin shows usually a normal dentinoid border, and, in so far as it constitutes a greater proportion of the tooth mass than normally, it strengthens the tooth beyond the normal. The parathyroids are therefore not playing a primary rôle in this problem, although they may very likely play an incidental rôle. In the present state of our knowledge we cannot entirely exclude the possibility that vitamins may also be involved, in spite of the fact that the animals received an adequate diet. Certain vitamins may not be utilizable.

Our histologic findings can be accounted for on the basis of, *a*) disproportion between the rate of tooth growth and rate of eruption (Gottlieb, '20); *b*) vascular disturbances. Either *a* or *b* may be the primary factor.

Disproportion in growth and eruption rates. Our finding that the rate of eruption is disturbed is based on histologic and quantitative data. Accepting Demolis' ('28) conception that eruption is normally brought about by forces that are traceable to tooth growth and to alveolar bone growth, the retardation of eruption of the incisor of the hypophysectomized rat is likely to be the sum total result of two factors; first, the retardation of tooth growth; secondly, the retardation of alveolar bone growth. Following hypophysectomy, there is an immediate and almost complete stoppage of skeletal growth (Smith, '30). This means that the alveolar bone is similarly checked and disturbed in its growth and cannot therefore respond and adjust itself normally to the tooth which tends to grow in length and in width and which in addition undergoes, as it matures, a flattening of its curvature (Erdheim, '14). Thus instead of normally aiding eruption, the retarded growth of the alveolar bone hinders eruption.

However, there is a formation and accumulation of some tooth substance even in the final stages when eruption has entirely ceased. This may indicate that the rate of tooth growth is less severely disturbed than the rate of eruption. This disproportion (between the rate of tooth growth and the rate of eruption) results in an intense crowding and folding of the growing tooth especially at its basal zone. This alone may produce an atrophy and reduction of blood supply.

Vascular disturbances. On the other hand, the striking disturbance, reduction and occasional disappearance of what is normally a characteristic and exceedingly rich blood supply point to the possibility that the primary factor in this process is a circulatory disturbance which in turn produces alterations in alveolar bone growth, tooth growth, and rate of eruption.

It is probable that both factors are active and establish a vicious cycle that leads to the terminal picture presented in figure 27.

SUMMARY

The effect of hypophysectomy on the incisor of the white rat has been studied in twenty-three completely hypophysectomized rats (63 to 459 days after operation) and sixteen litter-mate control rats in respect to gross changes (including x-ray findings and eruption rates) and microscopic alterations.

The gross findings were:

1. Progressive retardation and final cessation of eruption.
2. Distortion of form, especially in the basal zone.
3. Smaller size of teeth (two-thirds normal size).

The histologic findings were:

1. The enamel epithelium undergoes regressive changes and foldings which result either in vicarious aborted enamel formation in the labial periosteum or in hypoplastic areas of enamel. The ganoblasts become cuboidal and flat and finally degenerate. The papillae become irregular, proliferate into the labial alveolar periosteum where they become isolated in form of epithelial islands and finally undergo degenerative changes. In the anterior zone in animals that lived more than ten months after the operation, the enamel epithelium is entirely lacking.

2. The enamel in the anterior zone is usually normal except for occasional hypoplastic areas that are often associated with connective-tissue ingrowths. In the basal zone the enamel shows many variations from normal structure to defective structure and complete absence. Occasionally appearances similar to the bands of Retzius are seen.

3. The dentin in the anterior zone fills the pulp almost entirely so that the tooth contains practically as much dentin as does the normal control although the bulk of the tooth is one-third smaller than the normal. The structure in this zone is usually normal except for some pulpal inclusions. At the basal zone the dentin forms the principal bulk of the multiple foldings and varies structurally from normally calcified to very poorly calcified dentin and irregular uncalcified matrix.

4. The pulp is almost completely obliterated and dentified in the anterior zone. The pulpal slit that remains in this zone is for the most part necrotic and contains many calcified globules. In the basal zone the pulp takes on a more or less typical appearance, but its blood supply can be seen to be reduced in comparison with the rich blood supply of the control material.

5. The cementum is thicker than normal. It sometimes reaches five times the normal thickness. Its outline is sometimes disturbed in the basal zone.

6. The periodontal membrane is, as a rule, normal in structure, but its blood supply is below normal. It is uneven in width and contains an increased number of epithelial rests.

7. The labial alveolar periosteum has a reduced blood supply and is irregular in width. Occasionally it contains islands of bone that are near the enamel or in contact with the enamel.

8. Hertwig's sheath displays greater vitality than any other dental tissue. It is often displaced. An accumulation of eosin-staining fluid between the sheath and the pulp is sometimes observed.

The changes are progressive. The actual conditions in a given case naturally depend on the time elapsing between the operation and the death of the animal.

The material presented in this paper throws interesting lights on various phases of the growth and eruption processes of teeth.

LITERATURE CITED

- ADDISON, W. H. F., AND APPLETON, J. L. 1915 The structure and growth of the incisor teeth of the albino rat. *J. Morph.*, vol. 26, pp. 43-96.
- ASCHNER, B. 1912 Über die Funktion der Hypophyse. *Arch. f. die gesammte Physiol.*, Bd. 146, S. 1-146.
- ASCOLI, G., AND LEGNANI, T. 1912 Die Folgen der Exstirpation der Hypophyse. *Münch. med. Woch.*, Bd. 59, S. 518-521.
- BLACK, G. V. 1924 *Special dental pathology*. Third ed., Chicago, pp. 460-476.
- DÉMOLIS, P. 1928 The phenomena of dentition. *Dent. Cosmos*, vol. 70, pp. 188-190.
- DOWNES, W. G. 1930 An experimental study of the growth effects of the anterior lobe of the hypophysis on the teeth and other tissues and organs. *J. Dent. Research*, vol. 10, pp. 601-654.

- ERDHEIM, J. 1906 Tetania parathyreopriva. Mitteilungen aus den Grenzgebieten, Bd. 16, S. 632-744.
- 1911 Über die Dentinverkalkung im Nagezahn bei der Epithelkörperchentransplantation. Frankf. Zeit. f. Pathol., Bd. 7, S. 295-342.
- 1914 Rachitis und Epithelkörperchen. Denkschr. der math. naturw. Klasse der Kais. Akad. der Wissenschaften, Wien, Bd. 90, S. 363-683.
- GOTTLIEB, B. 1920 Schmelzhypoplasie und Rachitis. Wiener Vierteljahrschr. f. Zahnheilkunde, Heft 2, S. 71-96.
- HAMMETT, F. S. 1922 Studies of the thyroid apparatus. VII. A differential effect of thyroparathyroidectomy and parathyroidectomy of the incisor teeth of the albino rat. Am. J. of Physiol., vol. 62, pp. 197-201.
- JUNG, F. T., AND SKILLEN, W. G. 1929 Effects of thyroparathyroidectomy on the teeth of the rat. Proc. Soc. for Exp. Biol. and Med., vol. 26, pp. 589-590.
- KELLNER, E. 1931 Das Verhältnis der Zement- und Periodontalbreiten zur funktionellen Beanspruchung der Zähne. Zeitschr. f. Stomat., Bd. 29, S. 44-62.
- KRANZ, P. 1914 Innere Sekretion in Beziehung zur Kieferbildung und Zahnentwicklung. Dtsche. Zahnheilk. in Vorträgen, Heft 32, S. 3-104.
- MARSHALL, J. A. 1927 Dental caries and pulp sequelae resulting from experimental diets. J. Am. Dent. Ass., vol. 14, pp. 3-37.
- ORBAN, B. 1927 Nutrition and teeth. J. Am. Dent. Ass., vol. 14, pp. 1619-1630.
- OWEN, R. 1840-1845 Odontology. H. Baillière, London.
- ROMEIS, B. 1928 Taschenbuch der mikroskopischen Technik. R. Oldenbourg, München und Berlin, S. 134-135.
- SCHOUR, I. 1932 The teeth. In: Special Cytology, 2nd ed., sec. III, vol. 1, pp. 69-103. Paul B. Hoeber, Inc., New York.
- SCHOUR, I., AND VAN DYKE, H. B. 1932 The effect of replacement therapy on the eruption of the incisor of the hypophysectomized rat. Proc. Soc. Exp. Biol. and Med., vol. 29, pp. 378-382.
- TOYOFUKU, T. 1911 Über die parathyreoprive Veränderung des Rattenzahnes. Frankf. Zeit. f. Path., Bd. 7, S. 249-294.
- VAN DYKE, H. B., AND WALLEN-LAWRENCE, Z. 1930 On the growth-promoting hormone of the pituitary body. J. Pharmacol. and Exper. Therap., vol. 40, pp. 413-422.
- WALLEN-LAWRENCE, ZONJA, AND VAN DYKE, H. B. 1931 The gonad-stimulating substances of the anterior lobe of the pituitary body and of pregnancy-urine. J. Pharmacol. and Exper. Therap., vol. 43, pp. 93-124.

PLATE 1

EXPLANATION OF FIGURES

1 Drawing of hypophysectomized rat 113 showing enamel waves on the exposed labial surface of the upper incisors. Note the two dark enamel spots on the upper right incisor. $\times 1\frac{1}{2}$. This animal lived 459 days after the operation.

2 Photograph, showing the comparison in size of the right mandible of control rat 202 (above) and its hypophysectomized litter-mate 199 (below). $\times 1.8$.

3 Radiograph of upper incisor of hypophysectomized rat 113. Compare with figure 4. $\times 3$.

4 Radiograph of upper incisor of rat 112 which is the litter-mate control to rat 113. $\times 3$.

5 Radiograph of upper incisor of hypophysectomized rat 258, showing the distribution of the foldings and waves over the entire labial surface. $\times 3$. This animal lived 412 days after the operation.

6 Radiograph of basal zone of lower incisor of hypophysectomized rat 113. compare with figure 7. $\times 3$.

7 Radiograph of basal zone of lower incisor of rat 112 which is the litter-mate control to rat 113. $\times 3$.

8 Radiograph of upper incisor of hypophysectomized rat 151, showing distortion of its curvature. $\times 3$. This animal lived 380 days following the operation.

9 Radiographs of left and right halves of the head of hypophysectomized rat 314. The long axis of the upper incisors follows two arcs of two circles. The labial surface of the upper right incisor (below) shows an interruption, the histologic picture of which is shown in figure 11. Natural size. This animal lived 110 days after the operation.

10 Photomicrograph of a section of the labial surface of the anterior zone of the upper incisor of hypophysectomized rat 108 (a litter-mate to rat 113). $\times 184$. This animal lived 273 days after the operation. Note the absence of enamel epithelium. *Al.b.*, alveolar bone; *En.Sp.*, space formerly occupied by enamel lost in decalcification; *L.a.p.*, labial alveolar periosteum.

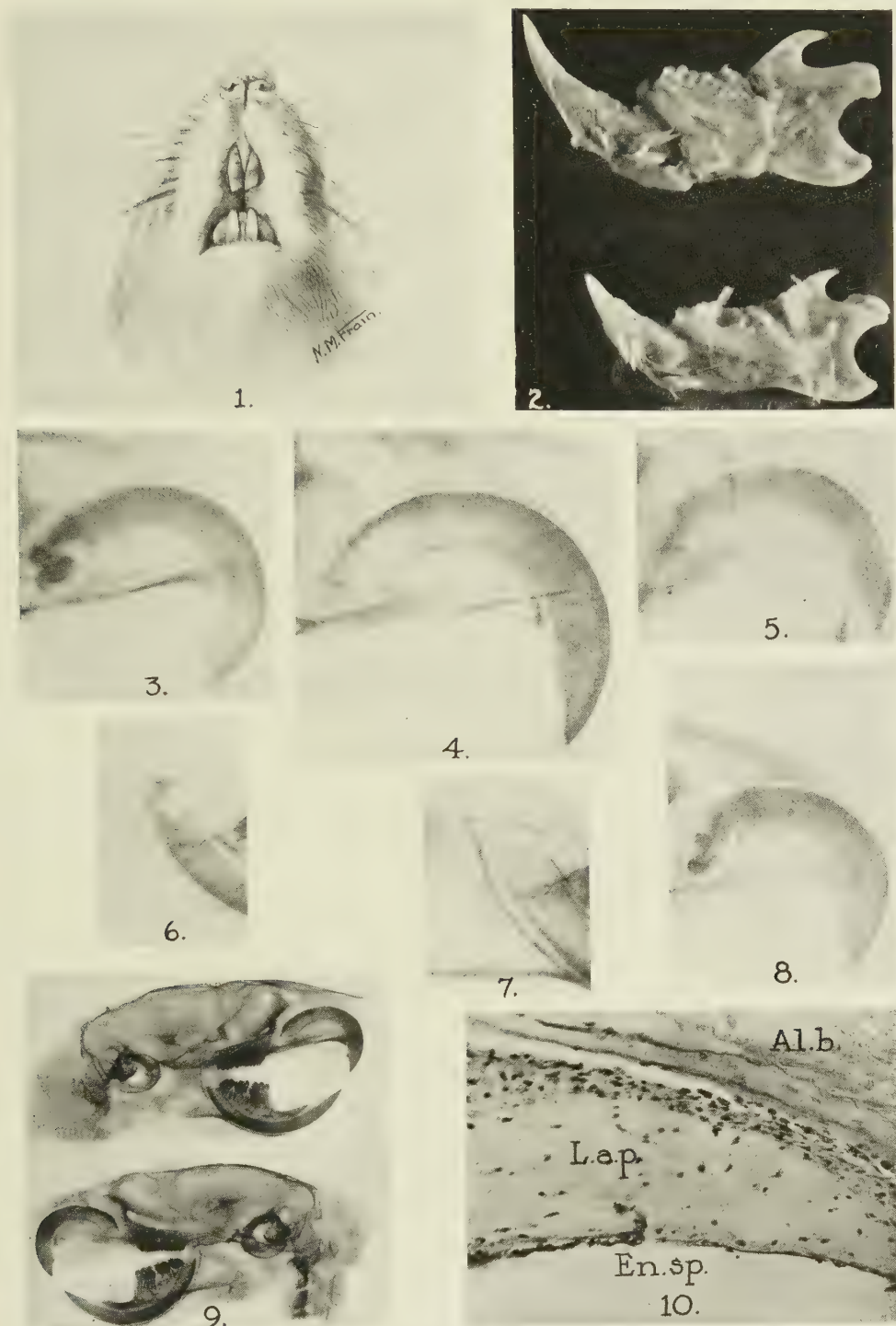


PLATE 2

EXPLANATION OF FIGURES

11 Drawing of the folding of the labial alveolar periosteum and the enamel epithelium indicated in the interruption of the labial surface of the upper right incisor shown in figure 9. $\times 375$ (approximate). *En.sp.*, enamel space; *Gan.*, ganoblasts; *L.a.p.*, labial alveolar periosteum; *Org.en.*, organic enamel matrix; *Pap.*, epithelial papillae.

12 Radiograph of head of control rat 202. $\times 2.1$.

13 Radiograph of head of hypophysectomized rat 144. $\times 2.1$. This animal lived 257 days after the operation.

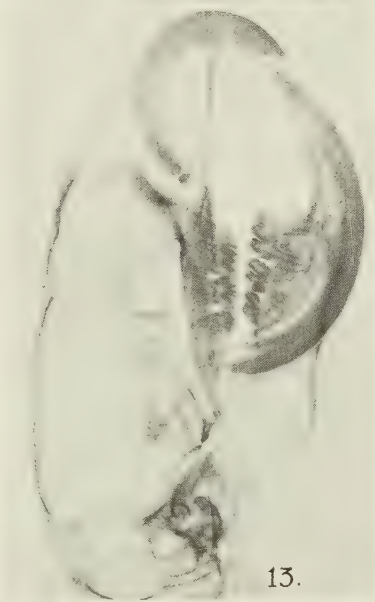
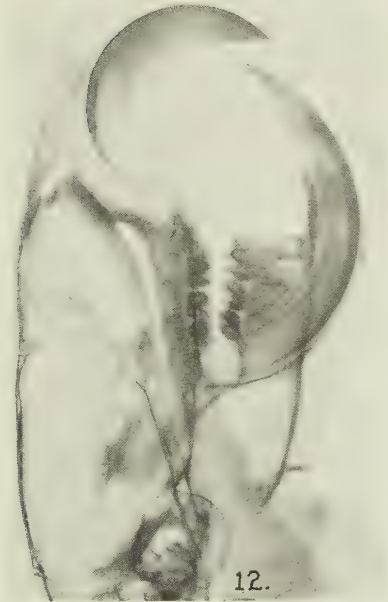
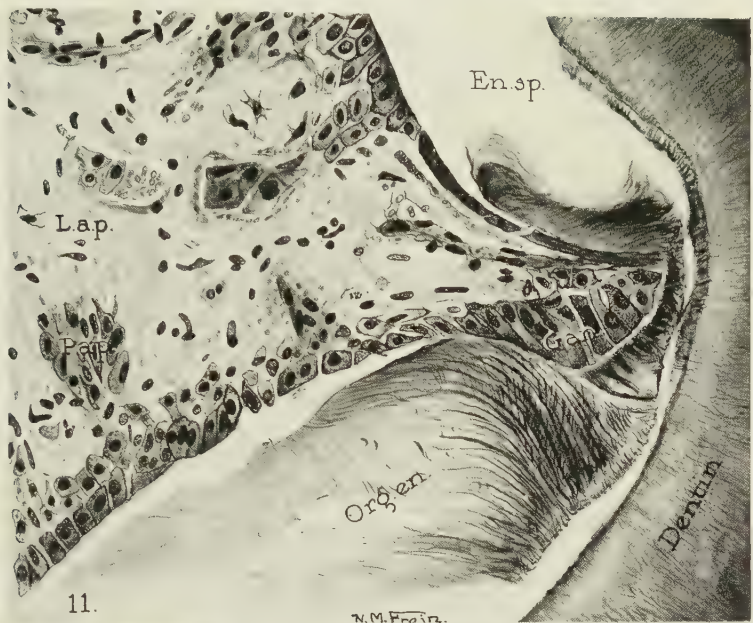


PLATE 3

EXPLANATION OF FIGURES

14 Photomicrograph of midsagittal section of the upper incisor of a normal mature rat. $\times 7.3$. Note the rich blood supply of the labial alveolar periosteum (*L.a.p.*). *Al.b.*, alveolar bone; *En.sp.*, enamel space; *GING.*, gingivae; *H.sh.*, Hertwig's sheath; *P.d.m.*, periodontal membrane.

15 Photomicrograph of midsagittal section of the upper right incisor of hypophysectomized rat 144. $\times 7.2$. Compare with figure 14. *Al.b.*, alveolar bone; *En.sp.*, enamel space; *Ging.*, gingivae. This animal lived 257 days after the operation.

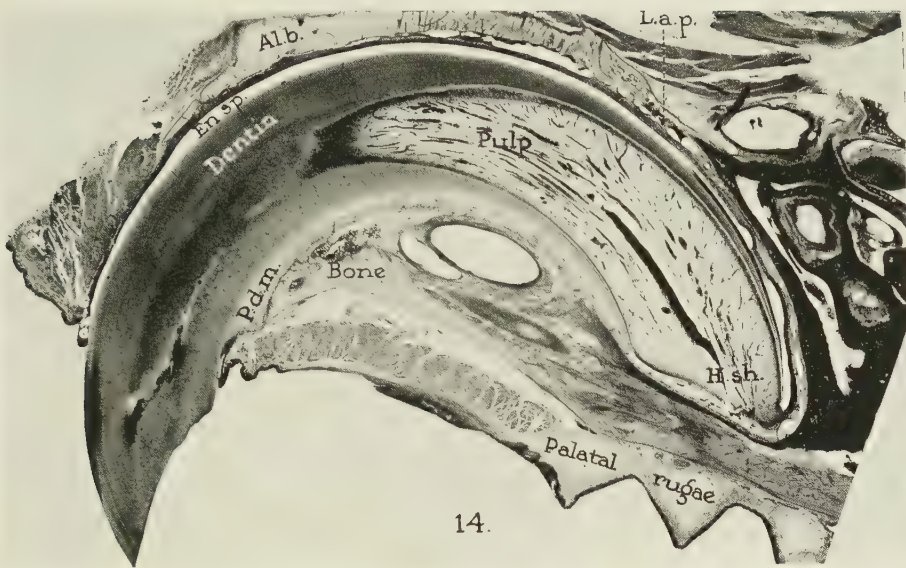


PLATE 4

EXPLANATION OF FIGURES

16 Photomicrograph of section from enamel epithelium of posterior region (immediately posterior to the folding shown in fig. 11) of labial surface of upper incisor of hypophysectomized rat 314. $\times 340$. This animal lived 110 days following the operation. Note the elongation and knob-like endings of the epithelial papillae. *En.sp.*, enamel space; *Gan.*, ganoblasts; *L.a.p.*, labial alveolar periosteum; *Pap.*, epithelial papillae.

17 Photomicrograph of section from enamel epithelium of anterior region (anterior to field of fig. 11) of labial surface of upper incisor of hypophysectomized rat 314. $\times 340$. Note the low cuboidal ganoblasts (*Gan.*) and the less numerous lower and irregular epithelial papillae (*Pap.*). *En.sp.*, enamel space; *L.a.p.*, labial alveolar periosteum.

18 Photomicrograph of section from enamel epithelium of middle portion of the labial surface of upper incisor of control rat 202. $\times 275$. *Al.b.*, alveolar bone; *En.sp.*, space formerly occupied by enamel lost in decalcification; *Gan.*, ganoblasts; *L.a.p.*, labial alveolar periosteum; *Pap.*, epithelial papillae.

19 Photomicrograph of section from enamel epithelium of posterior region (about 1 mm. from Hertwig's sheath) of the labial surface of the upper incisor of hypophysectomized rat 314. $\times 340$. This animal lived 110 days after the operation. *L.a.p.*, labial alveolar periosteum; *En.sp.*, enamel space; *Gan.*, ganoblasts; *Pap.*, epithelial papillae.

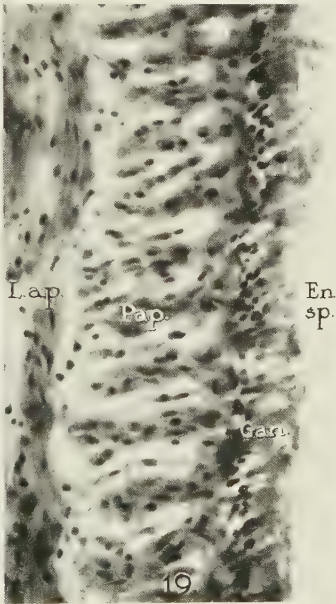
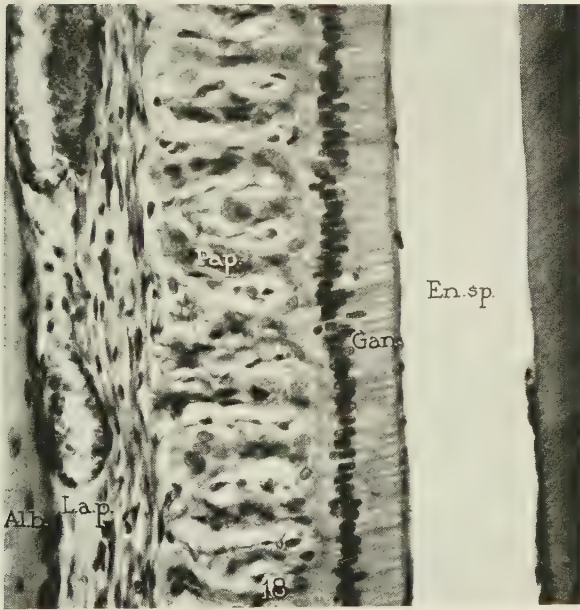
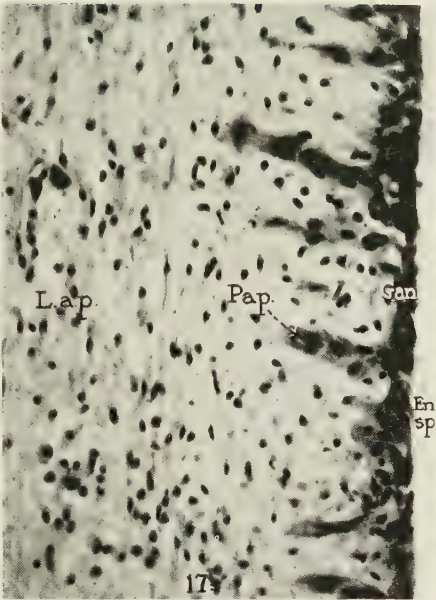
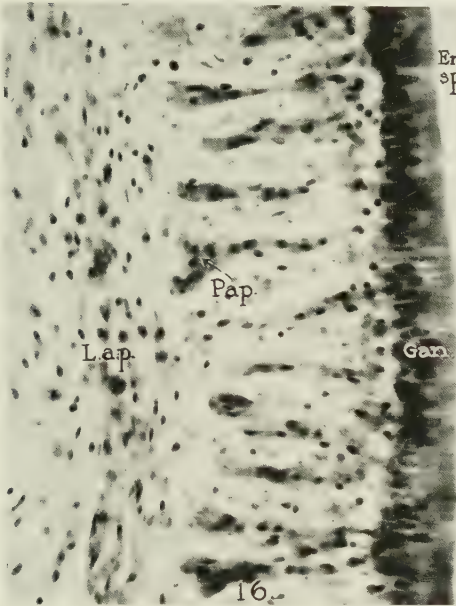


PLATE 5

EXPLANATION OF FIGURES

20 Photomicrograph of area indicated in figure 15 and magnified. $\times 165$. The labial alveolar periosteum (*L.a.p.*) contains isolated islands of epithelium (*Ep.isles*) showing all stages of degeneration. *En.sp.*, enamel space.

21 Photomicrograph of a ground section of the anterior portion of the labial surface of an incisor of a young normal rat. $\times 385$. This preparation was ground to preserve the enamel. Note the high columnar ganoblasts (*Gan.*) next to the outer enamel layer (*O.en.*). The inner layer of enamel (*I.en.*) shows decussating enamel rods. Stained with hematoxylin.

22 Photomicrograph of shallow folding of enamel and enamel epithelium of area indicated in figure 15 and magnified. $\times 120$. *En.sp.*, enamel space; *Gan.*, ganoblasts; *L.a.p.*, labial alveolar periosteum; *Pap.*, epithelial papillae.

23 Photomicrograph of area indicated in figure 22 and magnified. $\times 870$. Note the cuboidal form of the ganoblasts (*Gan.*) and the degeneration of the cells of the irregularly arranged epithelial papillae (*Pap.*). *En.sp.*, enamel space; *L.a.p.*, labial alveolar periosteum.

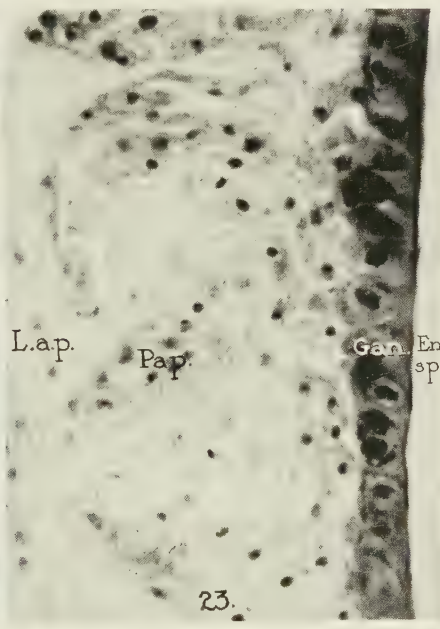
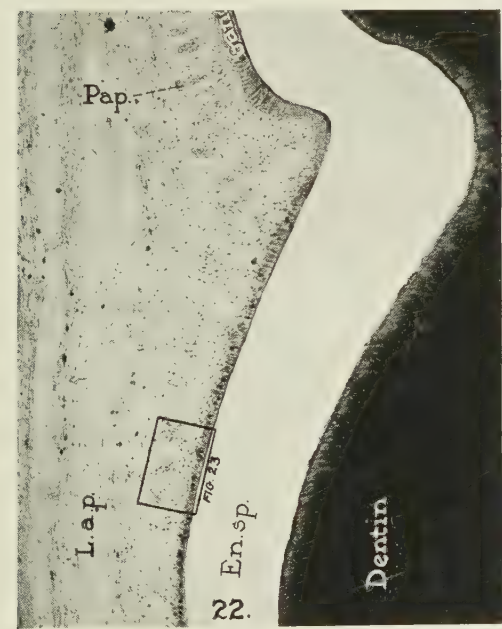
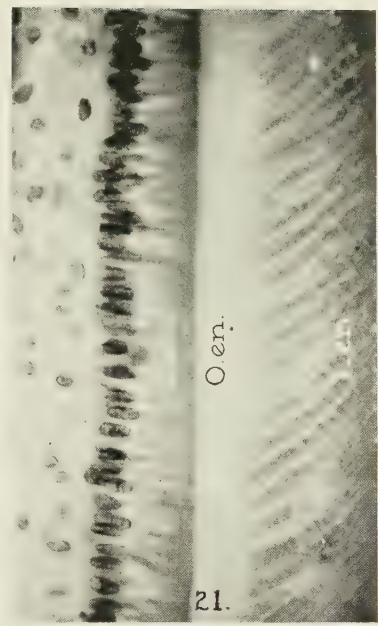
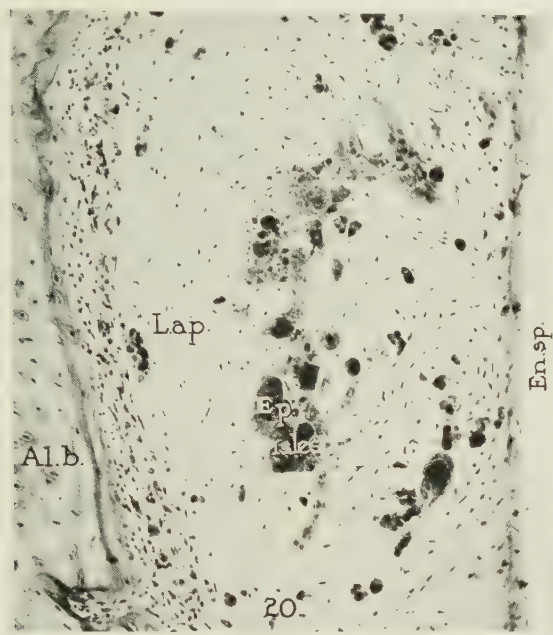


PLATE 6

EXPLANATION OF FIGURES

24 Drawing of region in anterior zone of upper incisor of hypophysectomized rat 111, showing an island of bone in the labial alveolar periosteum (*L.a.p.*) and new bone formation in direct contact with the organic remains of enamel (*Org.en.*). $\times 110$ (approximate). *Dent.*, dentin; *En.sp.*, enamel space. This animal lived 443 days following the operation.

25 Sketch of lingual dentin of the posterior zone of upper incisor of hypophysectomized rat 111. Note the irregular course of the cementum surface of the dentin. $\times 40$ (approximate). *P.d.m.*, periodontal membrane.

26 Photomicrograph of area indicated in figure 15 and magnified. $\times 90$. Note the resemblance to a section of a crown of an unerupted tooth. *Dent.*, dentin; *En.ep.*, enamel epithelium; *En.sp.*, enamel space; *Ep.sh.*, Hertwig's epithelial sheath; *Gan.*, ganoblasts; *L.a.p.*, labial alveolar periosteum; *Odont.*, odontoblasts; *Org.en.*, organic enamel matrix.

27 Photomicrograph of a midsagittal section of upper incisor of hypophysectomized rat 113. $\times 5.7$. Note the multiple deep foldings in the basal zone and the area of bone in the labial alveolar periosteum (*L.a.p.*). *Al.B.*, alveolar bone; *PD.M.*, periodontal membrane. This animal lived 459 days following the operation.

